Pluripotent stem cell research

Pioneering solutions and integrated workflows for induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs)
Pioneering solutions

Pluripotent stem cell (PSC) research is a continuously evolving field that beholds great promises for the future, opening up new opportunities in regenerative medicine. The great application potential of PSCs ranges from *in vitro* disease modelling and drug screening to translational research as foundation for clinical cellular-based therapeutic approaches.

We at Miltenyi Biotec think translational and want to support your PSC research with high-quality reagents, automated solutions, and 30 years of expertise.

**High-quality reagents**
We offer high-quality reagents and kits to support you in every step of your workflow. With our solutions tailored for PSC research, we cover tissue dissociation, cell isolation, cell cultivation, and phenotyping. Selected reagents are available in RUO and MACS® GMP grade for easy translation.

**Automated solutions**
Our instruments are designed to meet your research needs. From manual cell separators for small-scale experiments to fully automated high-content imaging.

**Expertise**
Discover our free step-by-step protocols, scientific posters, and watch-on-demand webinars. Join our face-to-face trainings in our MACS Academy.

PSC-derived brain organoid cleared with MACS® Clearing Kit and stained with anti-Ki-67 antibody (yellow), anti-βIII-Tubulin antibody (turquoise), and anti-Sox2 antibody (magenta). Picture was taken with UltraMicroscope II.
Workflows

4  iPSC reprogramming workflow
8  PSC culture and maintenance workflow
14 PSC differentiation workflow
21 Special focus: differentiation of PSCs into cardiomyocytes
iPSC reprogramming workflow

Cellular reprogramming of primary fibroblast cultures is the most common way to generate induced pluripotent stem cells (iPSCs). Discover our solutions for the iPSC reprogramming workflow and obtain viable and pure PSCs.
Easily obtain somatic cells for reprogramming

The Whole Skin Dissociation Kit, human was developed for the isolation of fibroblasts from diverse human skin biopsies. Used in combination with the gentleMACS™ Octo Dissociator with Heaters, it gently and efficiently dissociates human skin biopsies and generates fibroblast cultures from patient samples. Unlike traditional outgrowth cultures, it enables consistent monolayer cultures and yields enough fibroblasts for reprogramming within 5–8 days after plating.

- Developed for the isolation of fibroblasts from diverse human skin biopsies.
- No need to separate dermis and epidermis.
- Fast generation of fibroblasts in high numbers.

**Table 1:** Overview of gentleMACS Dissociators.

<table>
<thead>
<tr>
<th>Automation features</th>
<th>gentleMACS Dissociator</th>
<th>gentleMACS Octo Dissociator</th>
<th>gentleMACS Octo Dissociator with Heaters</th>
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<tbody>
<tr>
<td>Integrated heaters</td>
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<td></td>
<td>*</td>
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<tr>
<td>On-instrument enzyme incubation</td>
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<td>*</td>
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<tr>
<td>Fully automated protocols</td>
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<table>
<thead>
<tr>
<th>Sample processing</th>
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<tbody>
<tr>
<td>Number of sample positions</td>
<td>2</td>
<td>8</td>
<td>8</td>
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<tr>
<td>Parallel sample operation</td>
<td>*</td>
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<tr>
<td>Independent sample operation</td>
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<table>
<thead>
<tr>
<th>Software features</th>
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<tbody>
<tr>
<td>Pre-defined programs</td>
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<td></td>
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<tr>
<td>Free software update</td>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>User-defined programming</td>
<td></td>
<td></td>
<td>*</td>
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<tr>
<td>Program transfer between instruments.</td>
<td></td>
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</tr>
</tbody>
</table>

**Figure 1:** The unique combination of enzymatic treatment and mechanical disruption makes gentleMACS Technology the most gentle and convenient method for standardized and reproducible tissue dissociation.
Gently isolate PSCs

Having a homogeneous and high-quality PSC population is mandatory for any downstream experiment. After reprogramming, separation of iPSCs from un-reprogrammed cells is necessary to increase culture purity for further expansion. Moreover, isolating PSCs before starting a differentiation protocol will help to get consistent and reliable differentiation results.

Magnetic isolation of PSCs with MACS® Technology

Manual selection of PSCs can be highly subjective, technically difficult, and laborious. Taking advantage of our great experience in cell separation, we have developed easy-to-use strategies to obtain highly viable and pure PSCs.

Positive selection of PSCs
Isolate PSCs after reprogramming and before differentiation by positive selection with our MicroBeads detecting PSC markers. During separation, magnetically labeled PSCs are retained within the column, while unlabeled cells flow through. After a washing step, the column is removed from the magnetic field of the separator, and PSCs are eluted from the column.

• Short cell handling time.
• Highly pure populations.
• Cells maintain high viability.

Untouched isolation of PSCs
Alternatively, we offer solutions to deplete a PSC-containing culture from unwanted cell populations, such as fibroblasts after reprogramming procedure or feeder cells when switching from a co-culture to feeder-free conditions. Here, the unwanted cell type is magnetically labeled. During separation, the unlabeled target cells are collected in the flow-through fraction, while the unwanted cell type is retained within the column.

• Fast transition from feeder-based culture to feeder-free conditions.
• Efficient depletion of un-reprogrammed fibroblasts.

Figure 2: Positive selection with MACS Technology. Target cells are magnetically labeled. During separation, the magnetically labeled cells are retained within the column, while unlabeled cells flow through. After a washing step, the column is removed from the magnetic field of the separator and target cells are eluted from the column.

Figure 3: Untouched isolation with MACS Technology. Non-target cells are magnetically labeled. During separation, the unlabeled target cells are collected in the flow-through fraction, while non-target cells are retained within the column.
Products | Positive selection | Untouched isolation | Columns | Number of cells in total
---|---|---|---|---
Anti-TRA-1-60 MicroBeads, human | • | | MS or autoMACS® Columns | 2x10⁸
Pluripotent Stem Cell MicroBeads, human | • | | LS or autoMACS Columns | 2x10⁸
Anti-SSEA-4 MicroBeads, human | • | • | For positive isolation: LS or autoMACS Columns For depletion: LD or autoMACS Columns | 10⁹
Anti-Fibroblast MicroBeads, human | • | | LS or autoMACS Columns | 10⁹
Feeder Removal MicroBeads, mouse | • | | LS or autoMACS Columns | 10⁹

Table 2: Overview of MicroBeads for PSC isolation.

<table>
<thead>
<tr>
<th>Column Block</th>
<th>MS Columns, Large Cell Columns</th>
<th>MS Columns, Large Cell Columns</th>
<th>LS Columns, LD Columns</th>
<th>LS Columns, LD Columns</th>
<th>autoMACS Columns</th>
<th>Multi-24 Column Block, LS Columns, LD Columns</th>
</tr>
</thead>
</table>

Table 3: Overview of manual and automated MACS® Separators.
Consistent and high-quality PSC culture and maintenance are essential for reliable and reproducible results. Discover our products tailored to this workflow and optimize your culture conditions.
Culture is key

Balanced and carefully optimized media formulations are key features for maintaining high-quality PSCs. StemMACS™ iPS-Brew XF, human has been specifically designed to provide your PSC culture with the best-quality nutrients to sustain a robust growth and maintain a high pluripotent phenotype and differentiation potential. Its robust formulation is compatible with standard cell attachment matrices and allows you to choose different feeding schedules. Every-day feeding is over with StemMACS iPS-Brew XF, human!

- Xeno-free formulation, also available in MACS® GMP grade.
- Compatible with standard matrices.
- Weekend-free feeding schedule.

Figure 4: Phenotypes of hPSCs cultured in StemMACS iPS-Brew XF, human. Cells were assessed for the expression of key pluripotency-associated markers and analyzed by flow cytometry using the MACSQuant® Analyzer 10 (A). Cells show high expression of pluripotency markers and low expression of SSEA-1, a marker for early differentiation. Marker expression persists in a stable manner for over 20 culturing passages (B).

Our iPS-Brew GMP Medium has the same formulation as StemMACS iPS-Brew XF for easy translation.

Learn more about flexible feeding schedules with StemMACS iPS-Brew XF, human.

miltenyibiotec.com/ipsbrew

LEARN MORE
### Gently passage your PSCs

Use the StemMACS™ Passaging Solution XF for gentle detachment of PSC colonies and dissociation into cell clusters. The solution is designed to minimize manipulation of the culture, eliminating inactivation, dilution, or centrifugation steps. Thus, transfer into new cell culture conditions is reproducible, standardized, and fast, ensuring optimal viability and attachment.

- Preservation of cell-cell contacts (cluster passaging).
- Quick and simple protocol.
- Optimal viability and cell attachment.

### ROCK your passaging!

Enhance your PSC survival after passaging or thawing and prevent apoptosis by adding inhibitors of Rho-associated kinase (ROCK), such as StemMACS Y27632 or StemMACS Thiazovivin.

### Validation of PSC identity

PSCs must show specific characteristics both when establishing new ESC or iPSC lines, but also routinely during propagation of already established lines, in order to early detect signs of spontaneous differentiation.

A typical PSC characterization includes assessment of morphology, extra- and intracellular marker expression, pluripotent differentiation potential, and karyotyping. Most of these assays are time-consuming, tedious, and not suitable when working with high numbers of cell lines. Browse through our smart solutions to ease and fasten PSC characterization.

### Expression of pluripotency-associated markers: fast and comprehensive analysis with our multicolor panel for flow cytometry

Monitoring the pluripotency and differentiation status of PSC cultures has been done for long time by immunofluorescence microscopy. This method, however, is laborious and does not allow for reliable quantification of cell populations.

Discover our multicolor flow cytometry panel and benefit from:

- Simultaneous quantification of intracellular and surface markers.
- Qualitative and quantitative data.
- Excellent signal-to-noise ratio.

![Figure 5: Time-course of PSC morphology after passaging.](image)

The combined use of our culturing media and passaging reagents allows the maintenance of a typical PSC morphology independent of the splitting method (single-cell split or cell cluster split).

### Table 4: Multicolor flow cytometry panel for analysis of pluripotent stem cells.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Conjugate</th>
<th>Intracellular marker</th>
<th>Surface marker</th>
<th>Expressed in</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRA-1-60</td>
<td>PE</td>
<td>•</td>
<td>•</td>
<td>hPSCs</td>
</tr>
<tr>
<td>SSEA-4</td>
<td>VioGreen™</td>
<td>•</td>
<td>•</td>
<td>hPSCs</td>
</tr>
<tr>
<td>SSEA-5</td>
<td>VioBlue®</td>
<td>•</td>
<td>•</td>
<td>hPSCs</td>
</tr>
<tr>
<td>Sox2</td>
<td>FITC</td>
<td>•</td>
<td></td>
<td>hPSCs</td>
</tr>
<tr>
<td>Oct3/4</td>
<td>APC</td>
<td>•</td>
<td></td>
<td>hPSCs</td>
</tr>
<tr>
<td>CD15 (SSEA-1)</td>
<td>PE-Vio™ 770</td>
<td>•</td>
<td></td>
<td>Differentiated cells</td>
</tr>
</tbody>
</table>
**Figure 6: Multicolor flow cytometry analysis of undifferentiated human iPSCs.** Cells were stained with the antibodies as indicated and analyzed by flow cytometry on the MACSQuant® Analyzer 10. Unstained cells were used as a control for gating. Numbers in the heatmaps specify percentages of single-positive (bold numbers) and double-positive cells.

Learn how to perform multicolor flow cytometric analysis of hPSCs.
[mltenyibiotech.com/pscmcflow](http://mltenyibiotech.com/pscmcflow)
Functional assessment of pluripotency in just seven days

Pluripotency of PSC lines can be assessed in various ways, both in vitro and in vivo, via spontaneous or directed differentiation assays. The classical teratoma and embryoid body (EB) formation assays bear several limitations in terms of technical difficulty and duration of the assay itself.

Why would you work more to achieve the same result?

We have developed a standardized, quantifiable differentiation assay based on lineage-specific complete media, which supports directed 2D differentiation into all three germ layers within seven days. The StemMACS™ Trilineage Differentiation Kit, human allows for quantitative flow cytometric analysis as well as immunocytochemistry assessment.

- Ready-to-use media ensure reproducibility and minimize your effort.
- Side-by-side comparison of different cell lines or clones.
- Flexible analysis by immunofluorescence or flow cytometry.

Download our scientific poster on hPSC differentiation potential assessment.

miltenyibiotec.com/pscdiffposter

Figure 7: Workflow for pluripotency assessment with StemMACS™ Trilineage Differentiation Kit, human. Each hPSC line is seeded in three separate wells, one for each embryonic germ layer, and the assay can be started directly. Cells in each well are fed with StemMACS Trilineage EctoDiff Medium, MesoDiff Medium, or EndoDiff Medium respectively as illustrated. After seven days of culture, hPSC lines can be analyzed either by immunofluorescence staining or by flow cytometry. DRAQ5 (blue); PAX6, SM22a, and CXCR4 (red); Sox2, CD144, and Sox17 (green).
Cryopreservation of PSC lines is important not only as a good laboratory practice but also for the creation of large repositories and biobanks.

Use StemMACS Cryo-Brew for cryopreservation of PSCs to ensure high viability and rapid recovery after thawing.

- Chemically defined as well as xeno- and serum-free.
- High viability and cell recovery after thawing.
- For PSCs and PSC-derived cells.

**Antibody**

<table>
<thead>
<tr>
<th>Lineage</th>
<th>Antibody</th>
<th>Intracellular marker</th>
<th>Surface marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectodermal line</td>
<td>PAX-6 Antibody, anti-human, APC,</td>
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<td></td>
<td>REAfinity</td>
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<tr>
<td></td>
<td>Sox2 Antibody, anti-human/mouse,</td>
<td></td>
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<tr>
<td></td>
<td>FITC, REAfinity</td>
<td></td>
<td></td>
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<tr>
<td>Mesodermal line</td>
<td>CD114 (VE-Cadherin) Antibody,</td>
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<tr>
<td></td>
<td>anti-human, FITC, REAfinity</td>
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<tr>
<td></td>
<td>CD140b Antibody, anti-human,</td>
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<td></td>
<td>PE-Vio® 770, REAfinity</td>
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<tr>
<td>Endodermal line</td>
<td>CD184 (CXCR4) Antibody,</td>
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<td></td>
<td>anti-human, APC, REAfinity</td>
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<tr>
<td></td>
<td>Sox17 Antibody, anti-human,</td>
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<tr>
<td></td>
<td>Vio® B515, REAfinity</td>
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</table>

**Table 5:** Antibody panel for flow cytometric analysis of differentiated iPSCs.

**Figure 8:** StemMACS Cryo-Brew ensures high recovery and viability after cryopreservation of iPSCs.

Safely store your PSCs

Accurate analysis of functional pluripotency with our multicolor panel for flow cytometry

Our REAfinity™ Recombinant Antibodies allow for rapid but sensitive, qualitative, and quantitative analysis of hPSC differentiation potential. The antibody panel (table 5) consists of two antibody combinations for each embryonic germ layer (ectoderm, mesoderm, and endoderm) and detects both, surface and intracellular markers. This method allows for comparisons of different cell lines or clones.

**Get our step-by-step protocol for maintenance and freezing of PSCs.**

> miltenyibiotec.com/pscmainprot

> miltenyibiotec.com/pscdiffprot

Download the step-by-step protocol on flow cytometric analysis of the differentiation potential of hPSCs.
PSC differentiation workflow

Limit variability and increase standardization and efficiency in PSC differentiation. Discover our workflow and products.
Maintain consistency without losing flexibility

Our PSC differentiation portfolio offers you versatile but standardized reagents to grant flexible solutions with reliable results.

Many differentiations, one base medium

StemMACS™ DiffBase XF, human is a xeno-free and cytokine-free base medium for the differentiation of hPSCs. It has been developed to be used directly for hPSCs cultivated in StemMACS iPS-Brew XF, human and enables a smooth transition into the differentiation protocol without the need to adapt the cells to the new medium.

StemMACS DiffBase XF, human is so flexible that, if supplemented with the appropriate patterning cytokines and small molecules, it can be used as a base medium for virtually any type of differentiation. Its robust formulation supports cellular survival and successful differentiation of hPSCs both in monolayer-based differentiation protocols or tridimensional culturing.

Figure 9: StemMACS™ DiffBase XF, human supports directed differentiation in different culture conditions. Cultivation of hPSCs in StemMACS DiffBase XF, human supplemented with germ-layer specific patterning factors results in expression of early differentiation markers already after six days both in differentiation protocols based on monolayer adherent cultures (B) and in protocols based on 3D culture (A), as demonstrated by flow cytometric analysis. EB: embryoid body
StemMACS™ Small Molecules
The chemically defined nature of StemMACS Small Molecules ensures defined cell culture conditions and offers consistent biological activity with each lot. Thanks to their convenient ready-to-use in-solution format, the use is facilitated and error-proof.

- Defined cell culture conditions and consistent biological activity with each lot.
- Rigorously tested by HPLC and mass spectrometry.
- Detailed instructions for preparation of stocks.

MACS® Cytokines
To satisfy your needs, our recombinant cytokines and growth factors are available in three quality grades: research grade, premium grade, and MACS GMP Grade.

MACS Premium-Grade Cytokines are standardized recombinant proteins of highest quality and offer the convenience of well-defined biological activities.

- No lot-to-lot testing needed, saving time and costs.
- Apply the same amount of active cytokine every time for reproducible results.
- Efficient reagent usage without the need of oversaturation.

Using an embryoid body–based protocol?
Gently dissociate embryoid bodies for best results
Embryoid body (EB) formation is a crucial step in many ESC or iPSC differentiation protocols. Viable single-cell suspensions from EBs are a prerequisite for subsequent cell analysis or isolation and culture of specific cell populations.

The Embryoid Body Dissociation Kit, human and mouse was developed for standardized and reproducible dissociation of in vitro generated EBs or PSC-derived neurospheres. This convenient and time saving kit was optimized for high yields, high cell viability, and high reproducibility.

- Isolation of EB-derived cell populations using MACS Cell Separation Technology.
- Cultivation of EB-derived cells.
- Phenotyping or enumeration of individual EB-derived cell populations by flow cytometry.

Our recombinant cytokines are also available in MACS® GMP Grade for easy transition to clinical research.
Cell culture reagents for stem cell differentiation

**Pluripotent stem cell (PSC)**

- Activin A*
- CHIR99021

**Endodermal derivatives**

- **Definitive endoderm**
  - BMP-4
  - FGFR-2*
  - FGFR-3
  - Retinoic acid
  - Noggin
  - Retinoic acid
  - FGF-4
  - EGF
  - GDNF
  - IGF-1
  - SHH
  - BMP-7
  - FGF-2*
  - SB431542
  - CHIR99021
  - DAPT
  - SB431542
  - VEGF
  - LIF
  - IL-7*
  - SCF*
  - TPO
  - IL-3*
  - IL-6*
  - TPO

- **Neural crest**
  - Activin A*
  - BMP-4
  - CHIR99021
  - FGF-2*
  - GDNF
  - IGF-1
  - SHH
  - BMP-4
  - BMP-2
  - BMP-4
  - FGFR-2*
  - BMP-7
  - BMP-2
  - BMP-6
  - Dexamethasone
  - IGF-1
  - THP
  - IL-7*
  - SCF*
  - TPO*
  - IL-3*
  - IL-6*

- **Peripheral neuron**
  - BMP-4
  - G-CSF
  - RANK ligand
  - FGF-2*
  - BMP-7
  - BMP-2
  - BMP-6
  - FGF-2*
  - SCF*
  - IL-3*
  - IL-6*
  - TPO*

**Ectodermal derivatives**

- **Keratinocyte**
  - BMP-4
  - FGF-2*
  - IGF-1

- **Hair cell**
  - BMP-4
  - FGF-2*
  - IGF-1

- **Retinal pigmented epithelium**
  - BMP-4
  - FGF-2*
  - IGF-1

**Multipotent lung progenitor**

- EGF*
- HGF
- LIF
- PDGF-BB
- TGFB1*

**Mesodermal derivatives**

- **Adipocyte**
  - BMP-4
  - BMP-2
  - BMP-6
  - Dexamethasone
  - IGF-1
  - M-CSF
  - Rankeigand

- **Osteogenic cell**
  - BMP-2
  - BMP-6
  - Dexamethasone
  - IGF-1
  - M-CSF
  - Rankeigand

- **Chondrocyte**
  - BMP-2
  - BMP-6
  - Dexamethasone
  - IGF-1
  - M-CSF
  - Rankeigand

- **Common lymphoid progenitor**
  - SCF*
  - IL-7*

- **Megakaryocyte erythroid progenitor**
  - EPO
  - G-CSF
  - GM-CSF*
  - IL-3*
  - IL-6*

- **Common myeloid progenitor**
  - G-CSF
  - GM-CSF*
  - IL-3*
  - IL-6*

- **Cardiomyocyte**
  - BMP-2
  - BMP-4
  - PDGF-BB
  - SCF*
  - TPO*

- **Endothelial cell**
  - BMP-2
  - BMP-4
  - PDGF-BB
  - SCF*
  - TPO*

**Available from Miltenyi Biotec**

**Available also in MACS® GMP Grade**

**StemMACS™ Small Molecules**

**Product not available**
StemMACS™ CardioDiff Kit XF, human and StemMACS Cardiac Cultivation Medium XF, human have been designed to ease and standardize your PSC differentiation into cardiomyocytes and their cultivation. These easy-to-use media ensure high differentiation efficiency and do not require addition of supplements, allowing you to maintain a strong experiment-to-experiment consistency.

First, differentiate PSCs into cardiomyocytes in just eight days with StemMACS™ CardioDiff Kit XF, human.

- Fast and easy differentiation protocol.
- High differentiation efficiency.
- Scalable.

Then, hPSC-derived cardiomyocytes can be further cultivated in StemMACS Cardiac Cultivation Medium XF, human.

- Allows culture of PSC-derived cardiomyocytes for more than 30 days.
- Facilitates fast recovery after thawing.
- PSC-derived cardiomyocytes express characteristic markers and show typical morphology.

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**Figure 10: Timeline of hPSC-derived cardiomyocyte differentiation and cultivation.** Cardiac differentiation of hPSCs is induced stepwise by using three different media contained in StemMACS CardioDiff Kit XF, human. Specific fate-restricting media (Mesoderm Induction Medium, Cardiac Cultivation Medium, and Cardiac Induction Medium) are obtained just by mixing the appropriate supplement with StemMACS CardioDiff Basal Medium XF. After seeding a suitable amount of hPSCs, differentiation is commenced by adding the correct medium each day. If a longer cultivation of PSC-derived cardiomyocytes is required, differentiated cells can be maintained in StemMACS Cardiac Cultivation Medium XF, human.
Viable and homogeneous cell populations for reliable experiments

Working with homogenous cell populations is mandatory for reliable and reproducible experiments. Enriching your cell population before performing experiments allows you to:

- Obtain solid experimental results that come from your target PSC-derived cell population.
- Avoid interactions of different populations that might cause phenotypical changes.
- Control the purity of your cell population and keep it consistent within different experiments.

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Products</th>
<th>Strategy</th>
<th>Columns</th>
<th>Total cell number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectodermal lineage</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neural crest stem cells</td>
<td>Neural Crest Stem Cell MicroBeads, human</td>
<td>Positive selection or depletion</td>
<td>For positive selection: MS or autoMACS® Columns For depletion: LD or autoMACS Columns</td>
<td>(1 \times 10^9)</td>
</tr>
<tr>
<td>Glial progenitors</td>
<td>Anti-A2B5 MicroBead Kit, human and mouse</td>
<td>Positive selection</td>
<td>LS or autoMACS Columns</td>
<td>(1 \times 10^9)</td>
</tr>
<tr>
<td>Neural progenitors</td>
<td>Anti-PSA-NCAM MicroBead Kit, human and mouse</td>
<td>Positive selection</td>
<td>LS or autoMACS Columns</td>
<td>(1 \times 10^9)</td>
</tr>
<tr>
<td>Endodermal lineage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endodermal progenitor cells</td>
<td>CD184 (CXCR4) MicroBead Kit, human</td>
<td>Positive selection</td>
<td>LS or autoMACS Columns</td>
<td>(1 \times 10^9)</td>
</tr>
<tr>
<td>Mesodermal lineage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>CD144 (VE-Cadherin) MicroBeads, human</td>
<td>Positive selection or depletion</td>
<td>For positive selection: LS or autoMACS Columns For depletion: LD or autoMACS Columns</td>
<td>(1 \times 10^9)</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td>PSC-Derived Cardiomyocyte Isolation Kit, human</td>
<td>Depletion of non-myocytes and/or positive selection of cardiomyocytes</td>
<td>LS or autoMACS Columns</td>
<td>(2.5 \times 10^8)</td>
</tr>
</tbody>
</table>

Table 6: Highlighted products for target cell enrichment.
Fast and quantifiable solutions to evaluate the quality of your differentiation

PSC-derived cells must show cell type–characteristic phenotypes and functions. Take advantage of our comprehensive portfolio for flow cytometry, an easy and fast way to confirm the quality and phenotypical homogeneity of the cell culture.

Fast quality control during differentiation into dopaminergic neurons

The PSC-mDA Neuron Phenotyping Kit, human has been developed as flow cytometry–based quality control assay for in vitro phenotyping of the identity and purity of the culture during differentiation of PSCs into midbrain dopaminergic (mDA) neurons. Thanks to the parallel use of cellular controls, this kit allows for the detection of early expressed but specific regional markers and enables to assess cell identity and cell number of the different sub-populations, and to detect non-differentiated cells that might contaminate the culture.

- Qualitative and quantitative analysis.
- Fast in-process quality control assay.
- Based on REAfinitiy™ Antibody conjugates.

Interested in a specific antibody for your flow cytometry application? Browse through our website or check out our Custom Antibody Design Service.

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► miltenyibiotec.com/abberior

Figure 12: Schematic drawing and region-specific marker expression of a human fetal brain. During development, expression of region-specific markers arises in response to different concentration gradients of key patterning molecules. This differential marker expression allows to distinguish between different cellular populations and is at the base of the PSC-mDA Neurons Phenotyping Kit, human.

Figure 13: Example of flow cytometry–based quality control analysis with the PSC-mDA Neuron Phenotyping Kit, human. iPSC-derived mDA neurons were generated and analyzed after 16 days of differentiation. The kit contains antibodies against FoxA2, OTX2, PAX-6, TTF-1, Sox1, and Oct3/4 for characterization (see figure 12 for expected marker expression of mDA neurons). While remaining Oct3/4 expression hints to contamination of residual PSCs (staining 1), FoxA2, OTX2, and PAX-6 expression allow us to assess the presence of target mDA neurons (staining 2). Finally, TTF-1 and Sox1 expression reveal cell populations with a dorsal or caudal phenotype (staining 3).

Check out our application protocol on flow cytometry–based QC assay for PSC-derived midbrain dopaminergic neurons.

► miltenyibiotec.com/pscdopaprot
Special focus: Differentiation of PSCs into cardiomyocytes

Induction of differentiation
Ready-to-use media ensure highly efficient and reproducible differentiation of PSC-derived cardiomyocytes.

Culture
To help you meet your experimental needs, cardiomyocytes can be further cultivated or frozen.

Cell isolation
An homogeneous population of cardiomyocytes ensures reliable results.

Phenotyping
Fast and quantifiable solutions to evaluate the quality of your differentiation.

Figure 14: Immunofluorescence staining of mature PSC-derived cardiomyocytes. cTNT (green) and DRAQ5 (blue).

Discover our complete workflow for differentiation, isolation, and analysis of cardiomyocytes derived from hPSCs.

> miltenyibiotec.com/hpsccardioworkflow
Discover our instruments
Discover our instruments that support your PSC workflow. From tissue dissociators and cell separators over to flow cytometers and microscopes, our aim is to offer solutions tailored to your needs.

Discover our dissociators.
➤ miltenyibiotec.com/gentlemacs
Discover our cell separators.
➤ miltenyibiotec.com/separators
Discover our flow cytometers.
➤ miltenyibiotec.com/quant
Discover our imaging solutions and microscopes.
➤ miltenyibiotec.com/imaging

Product list

PSC reprogramming workflow

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<tr>
<th>Product</th>
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<tr>
<td>Whole Skin Dissociation Kit, human</td>
<td>130-101-540</td>
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<tr>
<td><strong>Cell separation</strong></td>
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<tr>
<td>Anti-TRA-1-60 MicroBeads, human</td>
<td>130-100-832</td>
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<td>Pluripotent Stem Cell MicroBeads, human</td>
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<td>Anti-SSEA-4 MicroBeads, human</td>
<td>130-097-855</td>
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<tr>
<td>Anti-Fibroblast MicroBeads, human</td>
<td>130-050-601</td>
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<td>Feeder Removal MicroBeads, mouse</td>
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PSC culture and maintenance workflow

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<td><strong>Maintenance Media</strong></td>
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<td>StemMACS™ IPS-Brew XF, human</td>
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<td><strong>Passaging</strong></td>
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<td>StemMACS Passaging Solution XF</td>
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<td>StemMACS Y27632</td>
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<td>StemMACS Thiazovivin</td>
<td>130-104-461</td>
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<td>associated markers by flow cytometry</td>
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<td>TRA-1-60 Antibody, anti-human, PE, REAfinity™</td>
<td>130-122-921</td>
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<td>**Characterization: Functional validation of</td>
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<td>StemMACS DiffBase XF, human</td>
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<td><strong>Characterization: Analysis of pluripotency</strong></td>
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<tr>
<td>StemMACS Cryo-Brew</td>
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## PSC differentiation workflow

### Induction of differentiation and culture
- **StemMACS DiffBase XF, human**
  - Order no.: 130-126-015
- **MACS® Neuro Medium**
  - Order no.: 130-093-570
- **MACS NeuroBrew-21 w/o Vitamin A**
  - Order no.: 130-097-263
- **StemMACS Cryo-Brew**
  - Order no.: 130-109-558
- **StemMACS Y27632**
  - Order no.: 130-103-922
- **StemMACS Thiazovivin**
  - Order no.: 130-104-461
- **Embryoid Body Dissociation Kit, human and mouse**
  - Order no.: 130-096-348
- **Neural Tissue Dissociation Kit (P)**
  - Order no.: 130-092-628
- **Neural Tissue Dissociation Kit (T)**
  - Order no.: 130-093-231

### Cell separation
- **Neural Crest Stem Cell MicroBeads, human**
  - Order no.: 130-097-127
- **Anti-A2B5 MicroBead Kit, human and mouse**
  - Order no.: 130-097-864
- **Anti-PSA-NCAM MicroBead Kit, human and mouse**
  - Order no.: 130-097-859
- **CD184 (CXCR4) MicroBead Kit, human**
  - Order no.: 130-100-070
- **CD144 (VE-Cadherin) MicroBeads, human**
  - Order no.: 130-097-857

### Cell characterization
- **PSC-mDA Neuron Phenotyping Kit, human**
  - Order no.: 130-127-439

## Special focus: PSC-derived cardiomyocytes differentiation

### Induction of differentiation
- **StemMACS CardioDiff Kit XF, human**
  - Order no.: 130-125-289

### Cell separation
- **PSC-Derived Cardiomyocyte Isolation Kit, human**
  - Order no.: 130-110-188

### Cell culture
- **StemMACS Cardiac Cultivation Medium XF, human**
  - Order no.: 130-125-287
- **Human Fibronectin (Fragment), premium grade, 0.1 mg**
  - Order no.: 130-109-392
- **Human Fibronectin (Fragment), premium grade, 1 mg**
  - Order no.: 130-109-393
- **StemMACS Cryo-Brew**
  - Order no.: 130-109-558
- **Multi Tissue Dissociation Kit 3**
  - Order no.: 130-110-204

### Cell characterization
- **Cardiac Troponin T Antibody, anti-human/mouse/rat, FITC, REAfinity**
  - Order no.: 130-119-674
- **α-Actinin (Sarcomeric) Antibody, anti-human/mouse/rat, FITC, REAfinity**
  - Order no.: 130-119-806
- **Myosin Heavy Chain Antibody, anti-human/mouse/rat, APC, REAfinity**
  - Order no.: 130-122-968
- **MLC2a Antibody, anti-human/mouse/rat, APC, REAfinity**
  - Order no.: 130-118-674
- **MLC2v Antibody, anti-human/mouse/rat, PE, REAfinity**
  - Order no.: 130-119-680

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